SLAC-SFA: U(IV) biomineralization and stability of monomeric U(IV) species

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In situ immobilization of uranium in a reduced form is the goal of bioremediation approaches. Additionally, reduced uranium is found associated with natural reduced zones at the Rifle IFRC. In the last year, we have expanded our work on the products of biological and abiotic U(IV) reduction in several ways. We probed the chemical species that led to preferential monomeric U(IV) formation, microscopically characterized biogenic formation of both products preserved in a frozen hydrated state, investigated the role of extracellular polymeric substances (EPS) and explored the *in situ* reactivity of monomeric U(IV) at the Rifle IFRC site.

The ratio of UO_2 to monomeric U(IV) produced by *Shewanella oneidensis* MR-1 is strongly dependent on the chemical composition of the medium in which U(VI) reduction occurs. Uranium L_{III} edge XAS and the wet chemical extraction technique we developed allowed the identification of PO_4^{3-} and Ca^{2+} as critical components promoting monomeric U(IV) formation. Surprisingly, the aqueous concentration of these solutes during U(VI) bioreduction remains constant, suggesting little binding of these solutes to biomass. This may suggest an indirect influence of the presence of phosphate and calcium in solution on biological properties of cells that, in turn, impact the product of U(VI) reduction.

Cryo-TEM images of high pressure frozen and cryo-sectioned *Shewanella oneidensis* captured during U reduction to form either UO_2 or monomeric U(IV) reveal discrete nucleation points on the surface of the outer membrane. Cryo-tomographic reconstructions of the same phenomena further confirm the exterior localization of electron-dense U. The 3D profiles contrast the nanocrystalline uraninite, observed in clumps and the monomeric U(IV), which forms tufts. Additionally, we are investigating the role of biomolecules in promoting the formation of monomeric U(IV) vs. UO_2 using scanning transmission X-ray microscopy (STXM). A protein core and phospholipid membranes typical of bacteria were identified along with additional lipids and polysaccharides representative of EPS located on the exterior of the cell. When U was localized in the same region using SEM/EDX, U could be detected in EPS-containing regions. Further work is ongoing to confirm these initial findings.

To study the reactivity of monomeric U(IV) in an aquifer, agarose gel pucks containing monomeric U(IV) were deployed in wells at the Rifle IFRC site for up to 3 months. Two types of monomeric U(IV) were studied: that associated with a *Shewanella sp.* isolated from the Rifle site, and monomeric U(IV) associated with a biogenic magnetite that was treated with phosphate prior to U(VI) reduction. U loss from monomeric U(IV) gel pucks is significantly faster than that from uraninite pucks. Digestion and X-ray absorption spectroscopy (XAS) analyses on the recovered gels reveals that the chemistry and oxidation state of the groundwater impacts the rate of U loss from the gels, as well as its oxidation and retention as U(VI) within the gels.

Monomeric U(IV) is an important research topic due to its relevance in the subsurface. Its formation is promoted by the presence of phosphate and calcium. Biomass-associated monomeric U(IV) occurs at discrete locations on the outer membrane and appears to co-localize with EPS. Finally, it is significantly more labile than nanoparticulate UO_2 *in situ*.